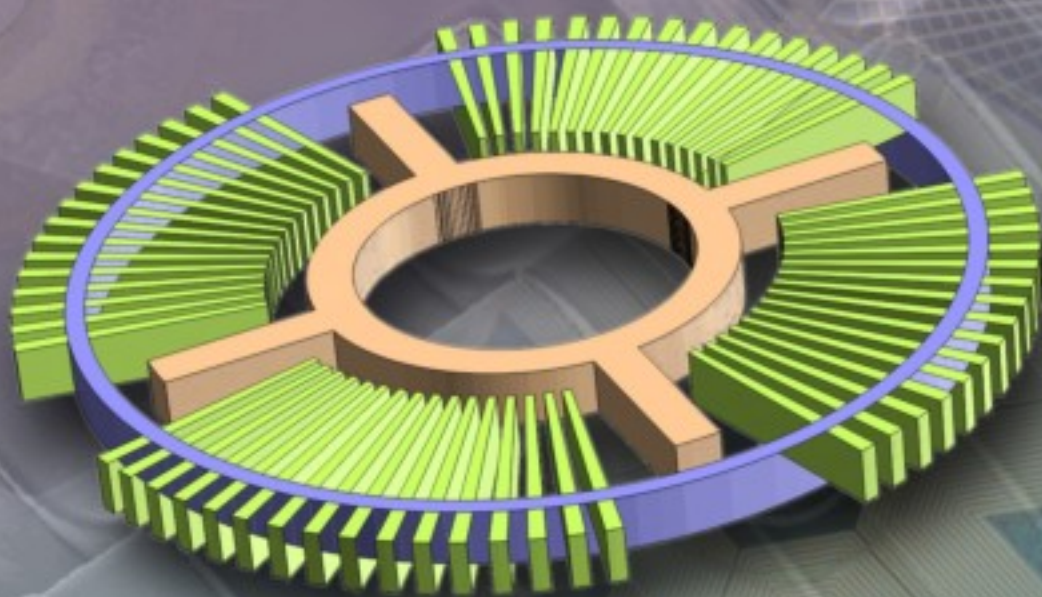


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Contents

Volume 3
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Research Articles

Foreword

Elena Gaura and James Brusey 1

Novel Synchronous Linear and Rotatory Micro Motors Based on Polymer Magnets with Organic and Inorganic Insulation Layers

Andreas Waldschik, Marco Feldmann and Stephanus Büttgenbach 3

Adaptive Subband Filtering Method for MEMS Accelerometer Noise Reduction

Piotr Pietrzak, Barosz Pekoslawski, Maciej Makowski, Andrzej Napieralski 14

Fluid-Dynamic and Electromagnetic Characterization of 3D Carbon Dielectrophoresis with Finite Element Analysis

Rodrigo Martinez-Duarte, Salvatore Cito, Esther Collado-Arredondo, Sergio O. Martinez and Marc J. Madou 25

Membranous Bypass Valves for Discrete Drop Mixing and Routing in Microchannels

Minsoung Rhee and Mark A. Burns 37

Ultrasound-driven Viscous Streaming, Modelled via Momentum Injection

James Packer, Daniel Attinger and Yiannis Ventikos 47

Multi-Functional Sensor System for Heart Rate, Body Position and Movement Intensity Analysis

Michael Mao, Bozena Kaminska, Yindar Chuo 59

NIR FRET Fluorophores for Use as an Implantable Glucose Biosensor

Majed Dweik and Sheila A. Grant 71

Electrostatic Voltage Sensors Based on Micro Machined Rotational Actuators: Modeling and Design

Jan Dittmer, Rolf Judaschke and Stephanus Büttgenbach 80

Optimization of Phage-Based Magnetoelastic Biosensor Performance

S. Huang, S.-Q. Li, H. Yang, M. Johnson, J. Wan, I. Chen, V. A. Petrenko, J. M. Barbaree, and B. A. Chin 87

Contribution of NIEL for Gain Degradation (β) in Si^{8+} Ion Irradiated Silicon Power Transistor

C. M. Dinesh, Ramani, M. C. Radhakrishna, S. A. Khan, D. Kanjilal 97

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Membranous Bypass Valves for Discrete Drop Mixing and Routing in Microchannels

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Abstract: A novel microfluidic component for discrete drop mixing in microchannels is presented. The membranous air bypass valve (MBV) in PDMS allows air to pass through it but stops liquid. Using the two-dimensional and three-dimensional MBVs, we removed the air between the discrete drops that were sequentially separated. The air removal then allows the drops to come in contact. The sequentially arranged drops then moved at a set velocity to achieve mixing. The resulting drop mixing occurs in three different regimes (diffusion-dominated, dispersion-dominated, and convection-dominated), depending on the Péclet number (Pe) and the drop dimensions. The time required to achieve sufficient mixing is controllable by changing the drop velocity, an operational parameter in the Péclet number. This MBV approach also enables precise control of the drop merging site. Experimental results for discrete drop mixing were in good agreement with the previous theoretical predictions. *Copyright © 2008 IFSA.*

Keywords: Drop mixing, Micromixer, Membranous bypass valve, Péclet number

1. Introduction

Rapid mixing in microfluidic systems is thus key to the effective functioning of biochemical processes in the devices [1-3]. However, in many microfluidic applications, rapid mixing is a major challenge because the time for diffusive mixing often exceeds processing times for other steps. This slow transport time scale can be thus a bottleneck for many high-throughput microfluidic assays.

Mixing is also one of the essential functions in lab-on-a-chip (LOC) platforms for complex chemical reactions [3-5]. Mixing in LOC microfluidic systems occurs not only in a continuous flow [6-8] but also must be controllable between two discrete drops in a batch operation. While most micromixers are used in continuous-flow systems, several studies have been reported on microfluidic mixing in batch systems [3-5, 9-10]. As opposed to continuous-flow systems, mixing in batch systems can be enhanced by convection [11]. During the course of drop transportation, the internal circulation streamlines of liquid in a moving discrete drop allows convective mixing as well as molecular diffusion. In addition, the isolated volume helps reduce axial dispersion along the channel and contamination between sample volumes [12].

We present here a novel microfluidic component to facilitate discrete drop mixing in microchannels. This new component helps remove the air between the discrete drops that are initially isolated in different places in the channel. The merged drop then is dislocated at variously controlled velocities to accelerate mixing. By experimental verification, three different regimes are classified and compared with the theoretical modeling. Additionally, a membranous one-way check valve is introduced. The valve prevents liquid flow until the internal pressure reaches at a certain threshold pressure. The threshold pressure can be adjusted by geometry and thus one can make the valve allow the flow in one direction while stops the flow in the opposite direction.

2. Materials and Methods

2.1. Device Fabrication

The prototypical mixing device was manufactured using the standard soft-lithographic technique [13]. The SU-8 (MicroChem) resist was spun on a bare silicon wafer to make the mold for fluidic channels and pre-baked on the hot plate for 5 min at the 65°C and for 20 min subsequently at the 95°C. After the exposure to 365 nm UV light, the coated wafer was post-baked for 1 min at the 65°C and for 10 min subsequently at the 95°C. The successive 15 min development resulted in the ~80µm thick mold. The depth and width of the channel were analyzed on a surface profiler (Alpha-Step 500, KLA-Tencor). A mixture of PDMS prepolymer and curing agent (5:1 w/w, Sylgard 184, Dow-Corning) was cast against the SU-8 mold and cured at 150°C for 18 min. The cured PDMS cast was carefully removed from the mold and diced into individual dies. For each die, four injection holes were drilled by an aluminum needle. Meanwhile, a glass cover slide (Dow Corning) was spincoated with a 10:1 PDMS mixture and cured to have a thin (~50µm) PDMS layer on the glass side. To bond the device and the glass lid, the PDMS replica and the PDMS-coated glass slide were then oxidized in the UV ozone cleaner (Jelight Company, Inc.) for 25 min and brought into conformal contact at 85°C in a convective oven for 2 hours.

2.2. Fluid Control and Image Acquisition

Each injection port in the device was connected through syringes to a computer-controlled setup, which consisted of four sets of two-way solenoid valves (Numatech). Each solenoid valve could perform a pulsed air pressure (<3 psi) injection or a pulsed vacuum suction. The switches to pressure and vacuum were programmed and operated by LabView (National Instruments). Liquid reagents were also loaded via the syringes with aid of the computerized pressure control. The experiments were performed on the device oriented on a stage of a stereomicroscope (Olympus SZX12). During the mixing progress, in situ imaging was recorded using a digital camera (Nikon Coolpix 4500) with a capture rate of 30 frames/s and then transferred to the computer for further analysis.

2.3. Image Processing

A blue dye (Trypan blue 0.4% solution, Sigma-Aldrich) and a colorless liquid (DI water) were used to characterize the mixing performance in this study. The luminance intensity images were recorded and transferred to the computer for evaluation. The RGB images captured were converted into grayscale images expressing only 256 levels of the luminance. In order to rectify light disturbance, a reference background picture was used and the grayscale values of the mixing pictures were subtracted from the background picture. A computer program was written to analyze the luminance levels of the pixels along a line drawn at the center across the mixing channel. In order to remove the high frequency noise, the finite impulse response (FIR) filter was employed during the image processing. The recorded images were analyzed in a grayscale mode, the background image was subtracted from the image to eliminate intensity fluctuations, and high frequency noise was removed by finite impulse response (FIR) filtering.

3. Results and Discussion

3.1. Membranous Air Bypass Valves

The operation to align two drops sequentially in microchannels is not straightforward. To aid in drop positioning and merging, we developed two different membrane bypass valve (MBV) in PDMS: two-dimensional and three-dimensional. Each valve consists of a thin ($\sim 30\mu\text{m}$) PDMS membrane facing the mixing channel and a separate bypass channel connected to atmosphere or a mild vacuum source. The membrane allows air to pass but not liquid. Fig. 1 illustrates the use of two-dimensional MBV to coalesce two drops. The trapped air between two drops can be either drawn off through the membrane by a vacuum source connected to the MBV or by applying air pressure from the both ends to push two drops towards the center and the trapped air out to atmosphere. Similarly, three-dimensional MBV is shown Fig. 2. The main mixing channel and the bypass channel are located in different layers, separated by a thin ($\sim 30\mu\text{m}$) PDMS membrane layer. The trapped air now will be aspirated into the above perpendicular channel where a vacuum source is connected.

The rate of air aspiration is dependent on the strength of vacuum connected to the bypass, the membrane thickness, the contact area of membrane interface, and the PDMS membrane porosity, which is affected by the mixing ratio of PDMS prepolymer and curing agent. Fig. 3 shows the influence of membrane interfacial area and the applied vacuum strength on the aspiration rate. For three-dimensional MBVs we can flexibly control membrane interfacial area by changing the shape of both mixing and bypass channels. However, the membrane in two-dimensional MBVs has limited variation in interfacial area, since the membrane height in two-dimensional MBVs is confined to the channel depth, typically less than $100\mu\text{m}$. While a two-dimensional MBV has its primary strength in more straightforward fabrication, it is less robust mechanically when high vacuum is applied.

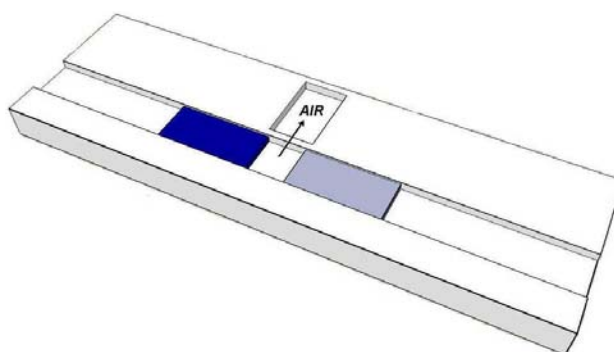
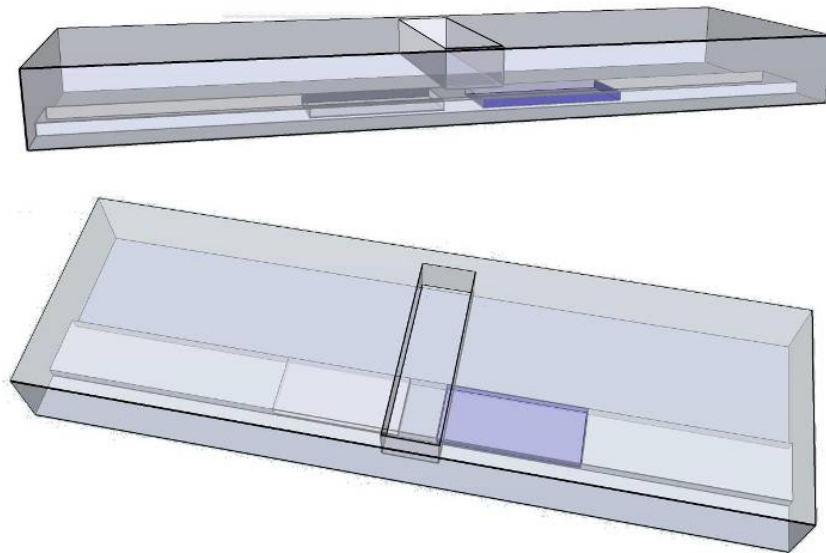


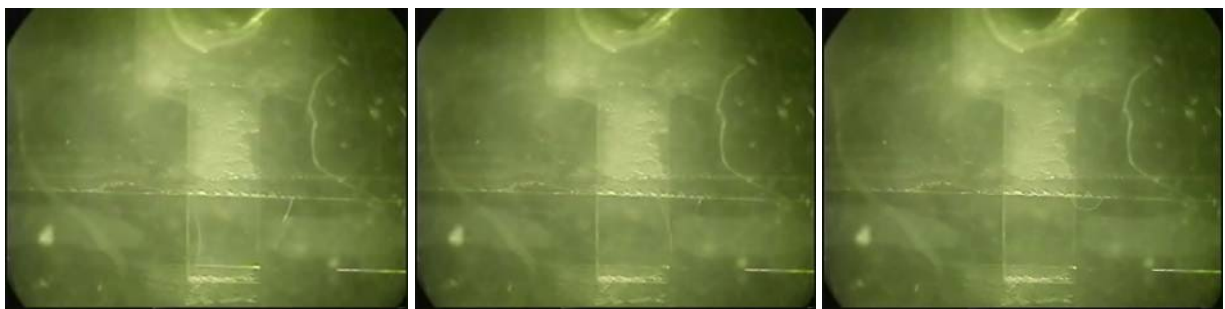
Fig. 1 (a). A schematic of the use of 2D MBV to coalesce two drops.



Fig. 1 (b). The trapped air between two drops can be drawn off by a vacuum source connected to the MBV or by applying air pressure from the both ends to push two drops towards the center and the trapped air out to atmosphere via the MBV.



(a)



(b)

Fig. 2. (a) A schematic of the 3D MBV to coalesce two drops viewed from the side and from the top, (b) The trapped air between two drops can be drawn off by a vacuum source connected to the MBV.

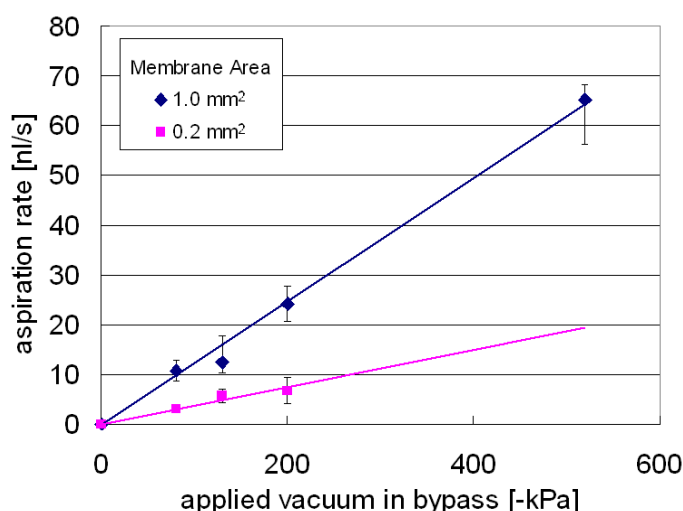


Fig. 3. The aspiration rate measurements with various membrane interfacial areas and the applied vacuum strength.

3.2. The Modified Péclet Number

The relative importance of convection to diffusion in mass transport is typically expressed as a dimensionless number, the Péclet number (Pe), where $Pe=U_d d/D$. Since the time required for sufficiently complete mixing will depend on the drop height (d) and velocity (U_d) as well as the diffusion coefficient of the solute (D), the Péclet number is useful to estimate the mixing time. To better explain the correlation between mixing and the aspect ratio, a modified Péclet number, has been suggested in our previous study [14]. When convective mixing occurs, convection and diffusion usually develop in different directions. Thus, for a drop with a high aspect ratio, the Péclet number provides insufficient information about the importance of convection and diffusion, mainly because the drop length (L) is not considered in the Péclet number. The modified Péclet number is defined by,

$$Pe^* = \frac{\text{diffusive time scale}}{\text{convective time scale}} = \frac{d^2/D}{L/U_d} = \frac{Pe}{\varepsilon} \quad (1)$$

Once the discrete drops get coalesced using the MBV, one can move the merged drop along the mixing channel at a set velocity to enhance mixing. For a given micromixer with fixed channel geometry and drop dimensions, one can alter the mixing condition (Pe^*) by controlling the drop velocity.

3.3. Drop Mixing in Three Different Regimes

The experimental mixing progress at a low Pe^* (~ 0.4) is shown in Fig. 4a, starting from the initial state when the two drops fuse and an interface develops ($t=0$ sec), to the state of complete mixing when the drop has traveled along the mixing channel. Figure 4a shows how the concentration of the merged drop changes during the mixing process when diffusion is dominating the mixing. The blue solid line represents the average concentration of the left domain that was initially occupied by the blue ink ($c=c_{\max}$), and the green dotted line shows the average concentration of the right domain initially without the blue dye ($c=0$). The merged drop has been displaced 63 mm for ~ 10000 seconds back and forth along the channel but the mixing was quite incomplete. The extended time required for complete mixing can result in a significant loss of drop volume due to evaporation, since the drop will be exposed to air through the vapor-permeable PDMS top for hours. In addition, reduction in drop volume makes the

concentration higher, possibly leading to inaccurate analysis of concentration data. The volume loss may be reduced by saturating the device environment with excessive water that covers the device top as well as the sample inlets. The resulting sacrificial evaporation of the supplied water fairly reduces the sample evaporation rate, but approximately 10-20% volume loss was still observed during the experiment.

In the intermediate range of Pe^* (~ 6) (Fig. 4b), however, the mixing occurs faster, and a different mixing mechanism, well-known Taylor dispersion, is observed. Because of this dispersive spreading, the mixing is greatly enhanced compared to purely diffusive mixing. Note that the concentration change for the dispersion-dominated mixing conforms to the behavior of diffusive transport but with a shorter timescale. When the modified Péclet number is small (Fig. 4c), the merged drop travels fast and rapidly mixes within a few seconds due to the convective effects. In Figure 4a, the average concentrations of both domains are oscillating, and gradually converging due to the internal circulation and the concentrations gradually converge with time until complete homogenization. The presence of this oscillation confirms the convection-dominated mixing at $Pe^*=132$.

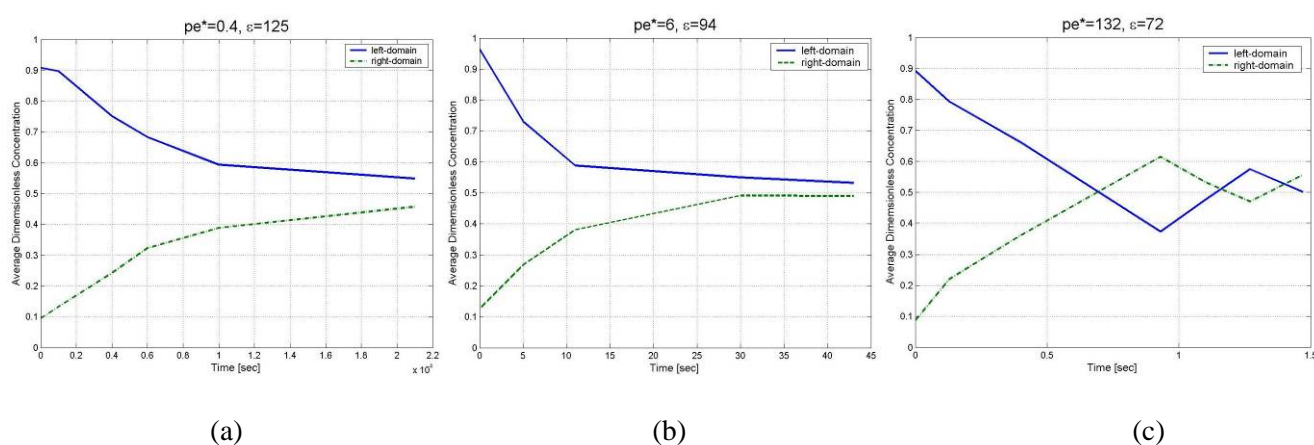


Fig. 4. Average concentration changes in left and right domains (a) Diffusive Mixing Progress ($Pe^*=0.4$), (b) Dispersive Mixing Progress ($Pe^*=6$), (c) Convective Mixing Progress ($Pe^*=132$).

3.4. Comparison of Mixing Experiment Results with Theoretical Modeling

Fig. 5 shows the plot of mixing time against the modified Péclet number for comparison of our experimental results with estimated curves from the theoretical modeling in our previous study [14]. Although the results are overall in good agreement with the theoretical modeling, it is important to note the slight discrepancy between the theoretical modeling and the experiment results. The underestimated mixing time (shorter mixing time than the theory predicts) is most likely due to the drop fusion mechanism using the MBV. At the moment when two discrete drops merge, they are drawn to each other and thus forced to collide, causing the influence of convective drop transport. Therefore, the fused drop is prone to make a deeper interface, indicating a slight degree of mixing has been already begun in the interfacial area leading to the underestimation of mixing time. This pre-mixing can mislead the measurements for slow diffusive mixing, especially. On the other hand, the overestimated mixing time (longer mixing time than the theory predicts) may be attributed to the air control. For the pressure-driven flow in the mixing experiments, the pressure from one inlet was generated in a pulsed manner, and it is likely that the measured mixing time would include non-mixing time between pulses.

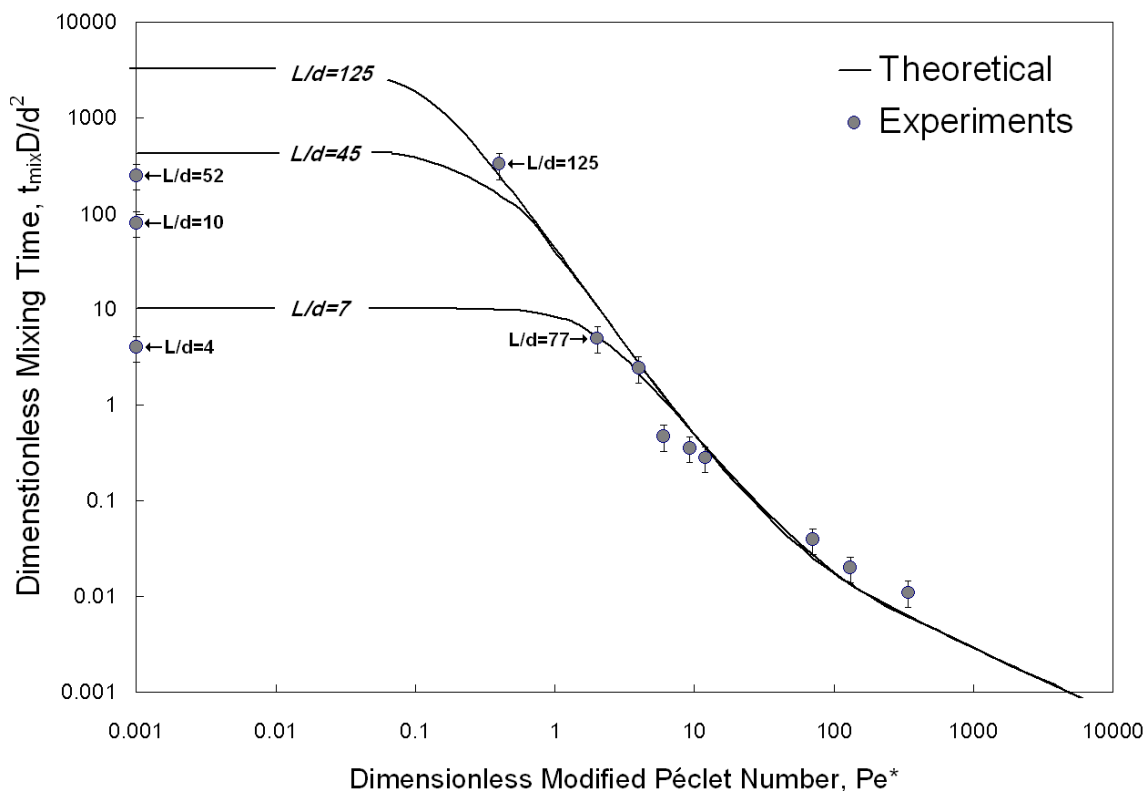


Fig. 5. Mixing time comparison among theoretical predictions, simulations, and experimental results.

3.5. Membranous Check Valves for Drop Routing

A one-way or check valve has become a concern in many microfluidic applications to perform tasks where backflow must be not allowed with minimal external operations. The microfluidic check valve is thus analogous to an electronic diode in function. While an electronic diode prevents current flow in one of the two directions in circuits, the fluidic check valve stops fluid flow in one direction. There have been many researchers who developed such valves that limit flow in one direction. Nevertheless, the use of such valves can be limited, since they involve a very complicated fabrication procedure due to their multi-layered design [15, 16] or the use of non-conventional materials [15, 17, 18].

Besides its unique bypass functionality, the MBV can also function as a check valve beyond its regular operational pressure (<2 psi). Depending on the pressure and membrane dimension, the membrane can be lifted and allow liquid to pass (Fig. 6a). When the low pressure gets recovered, the membrane returns to its upright position (Fig. 6b).

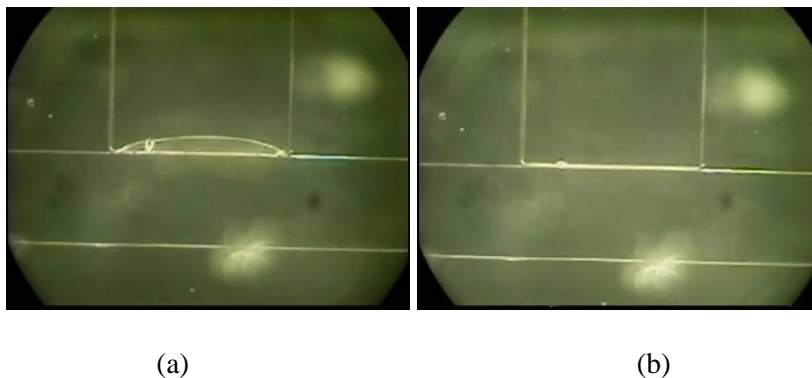


Fig. 6. Membranous Valve at different pressures applied: (a) open at >3 psi (b) closed at <1 psi.

Fig. 7a shows that a drop of blue ink penetrates the membrane when high enough pressure has been applied. The threshold pressure can be defined as the minimum pressure applied to the membrane that can open the membrane. The shape of membrane significantly affects the threshold pressure. For instance, a round-shaped membrane in Fig. 7b has a lower threshold pressure for the forward flow but a higher threshold pressure for the backward flow. In Fig. 8, at a chosen appropriate pressure, the liquid could pass through the upper valve but not the lower valve, since the internal pressure was high enough to open the upper valve while the lower valve was still closed. This design using multiple check valves with different threshold pressures may allow for selective routing of liquid samples around in the device. For 20 μm thick membranes, the flow rates were plotted against the pressure applied to the membrane, depending on the shape of membranes (Fig. 9). The threshold pressures are observed when the flow rate is being recovered to conform its original relationship, $Q=\Delta P/R$, from zero.

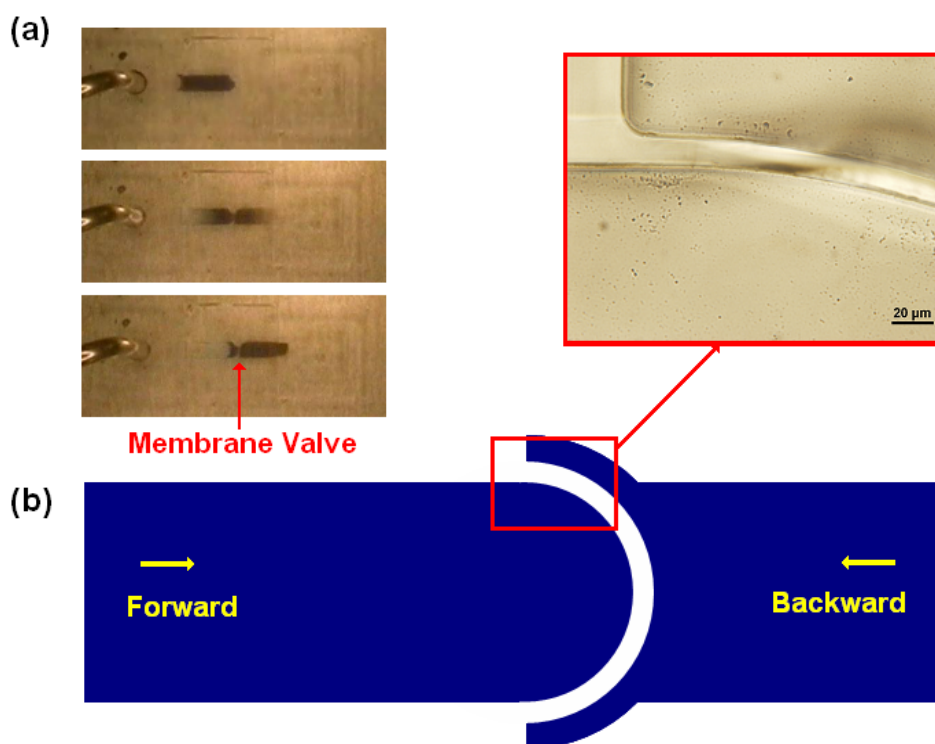


Fig. 7. (a) A drop of blue ink penetrates the membrane check valve when high enough pressure has been applied. The device was assembled with microfluidic assembly blocks [19]; (b) A round-shaped membrane of the thickness of 20 μm . The valve exhibits a lower threshold pressure for the forward flow but a higher threshold pressure for the backward flow.

The threshold pressure can be also increased by implementing a thicker membrane up to <50 μm . However, the membrane valve thicker than ~50 μm will not open without breaking the entire device, since the inherent bonding between the PDMS device and the substrate can withstand the threshold pressure of only 30-40 kPa inside the device.

The membranous check valve is a single-layer, planar device that can be fully integrated with standard multi-layer soft lithography techniques. Unlike previously reported multi-layered check valves, this simple planar component can be freely arranged in any location and thus provide many design options in developing flexible microfluidic systems.

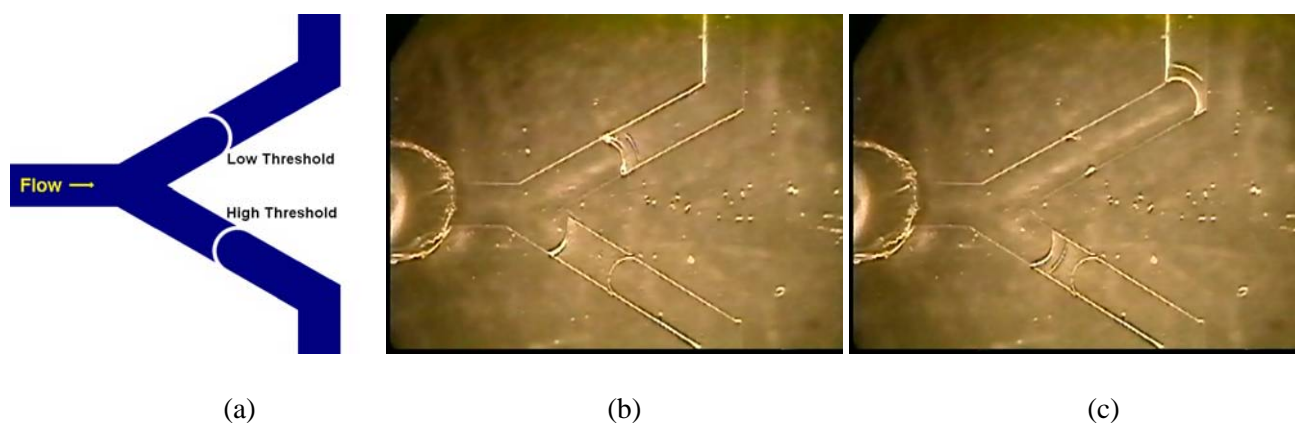


Fig. 8. Selective Routing. (a) The route design with two check valves with different thresholds. (b-c) The liquid sample will continue past the upper low threshold valve but not the high threshold valve. At higher pressure, the drop will flow into both directions.

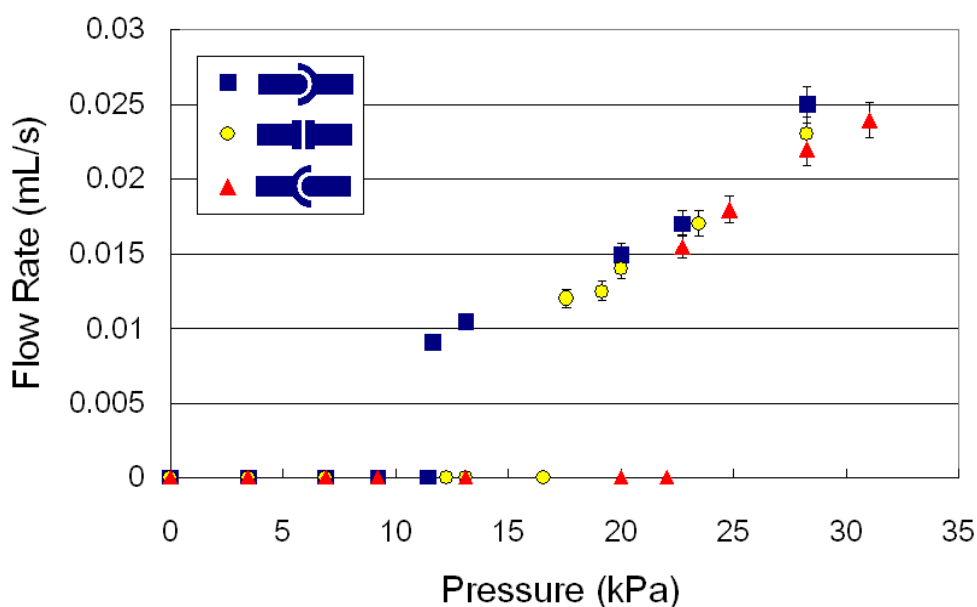


Fig. 9. A plot of flow rates against the internal pressure, depending on the shape of membranes.

4. Conclusions

The membranous air bypass valve (MBV) structures were constructed and verified with mixing experiments. In lab-on-a-chip systems, merging two drops separated by trapped air is a challenging task. We developed two different membrane bypass valves (MBVs) in PDMS: two-dimensional and three-dimensional, respectively. The membrane in PDMS is air-permeable but prevents liquid leakage. Using the MBVs, we successfully removed the trapped air between the discrete drops and let the fused drop move at a desirable velocity to achieve effective mixing. Experimental mixing results using the MBV were in good agreement with the previous theoretical predictions. Additionally, the membranous check valve structure was investigated with routing examples. At appropriate pressures, the liquid could pass through the check valve but not at lower pressures. The planar nature of such check valves makes them particularly useful for full integration with other complicated microfluidic components.

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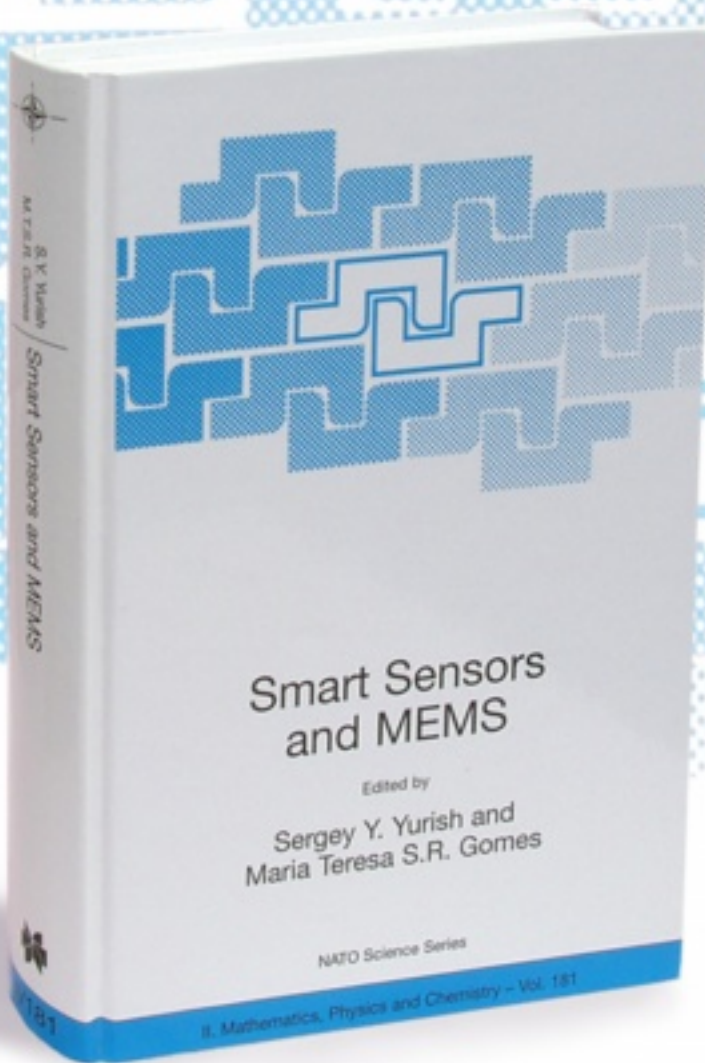
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